

# Detritus processing in lentic cave habitats in the neotropics

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Academic editor: M. Cobolli | Received 27 September 2012 | Accepted 11 March 2013 | Published 29 April 2013

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**Citation:** Souza-Silva M, Rezende KSR, Ferreira RL (2013) Detritus processing in lentic cave habitats in the neotropics. Subterranean Biology 11: 3–14. doi: 10.3897/subtbiol.11.5107

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## Abstract

Lentic cave habitats are almost always heterotrophic habitats where there are food and oxygen input from the surface. This hydrological exchange seems to be the key factor shaping most groundwater communities. Litter processing in cave water environments has not been experimentally studied as much as it has in lotic subterranean systems, although detritus is likely a critical resource for organisms inhabiting shallow groundwater habitats. The present study sought to evaluate the processing rates and the nitrogen and phosphorous dynamics in plant debris deposited in lentic habitats of two Neotropical limestone caves during 99 days. 84–10×10 cm<sup>2</sup> litterbags with mesh sizes of 0.04 mm<sup>2</sup> and 9 mm<sup>2</sup> were used. In each weighed litter bag, 50 green, intact plant leaf disks (± 2.0 gr/bag) were conditioned. At the end of the experiment, the average weight loss was only 17.4%. No macroinvertebrates were found associated to the debris, but significant differences in the processing rate in relation to the cave and mesh size were observed. The weight loss rate of the plant debris was considered slow (average 0.003 K<sup>-day</sup>). The amount of nitrogen and remaining phosphorous in the plant debris in the two caves showed variations over time with a tendency to increase probably due to the development of microorganisms which assimilate nitrogen and phosphorus. The slow processing rate of the plant debris can be due mainly to the fact that these lentic cave habitats are restrictive to colonization by shredder invertebrates. Furthermore, the abrasive force of the water, which plays an important role in the processing and availability of fragmented debris for colonization by microorganisms, is absent.

## Keywords

Caves, decomposition, plant debris, nutrients



## Introduction

The groundwater systems are heterotrophic habitats where food and oxygen availability are determined by importation from the surface. This hydrological exchange seems to be the key factor shaping most groundwater communities. Together with oxygen and organic matter, stygoxene and stygophile faunas also have access to the groundwater. If the food and oxygen supply are sufficiently high, these species are able to durably colonize the groundwater and compete with stygobites (Sket 1999; Hahn 2006).

The resource supply in cave ecosystems can vary greatly temporally in the same cave and among caves within a limited geographic area and in some cases can be similar to that in many surface stream types (Souza-Silva et al. 2012; Venarsky et al. 2012). Factors that contribute to the variability in organic matter abundance among cave ecosystems are unknown, but are likely to be a combination of landscape features (e.g. epigean standing-crop, doline topology, watercourse dynamics and plant community structure), cave structure (e.g. depth of cave and size of voids and fractures in the surrounding bedrock) and climate (e.g. precipitation and hydrology) (Souza-Silva et al. 2012; Venarsky et al. 2012).

However, there is a larger movement of leaves and trunks in the epigean environment as well as in the hypogean environment in periods of intense rain, when the water speed and flow are increased (Souza-Silva et al. 2011). Inside the caves, the plant fragments are deposited along the water courses constituting organic matter deposits that are slowly decomposed by detritivore invertebrates (Souza-Silva et al. 2012).

The decomposition or processing of debris occurs in successive degradation stages with the consequent release of nutrients such as nitrogen and phosphorous. These stages are influenced by the activity of physical-chemical agents such as current speed, temperature, pH, oxygen, dissolved nutrient content and the present plant or animal tissues, and also by the biological activity such as the meso- and microorganism actions (Allan and Castillo 2007).

Decomposition of leaf litter has been widely investigated in both aquatic and terrestrial environments (Gessner et al. 1999; Hattenschwiler et al. 2005; Sangiorgio et al. 2010), but few studies have addressed decomposition of leaf litter in aquatic environments characterized by low water velocity such as the river ponds (Chergui and Pattee 1990; McArthur et al. 1994; Baldy et al. 2002) or in very small isolated environments such as springs, pools and caves (Horton and Brown 1991; Eichen et al. 1993; Souza-Silva et al. 2007; Sangiorgio et al. 2010).

The present study aims to evaluate the processing rates of the coarse particulate organic matter and both nitrogen and phosphorous dynamics in plant debris exposed to decomposition in the lentic habitats in two Neotropical limestone caves.

## Materials and methods

### Study site

The study was carried out from April to July of 2009 in the Brega and Santuário limestone caves (Brega - 41°44'15"E, 77°42'20"S, elevation 725 m and Santuário



419312.50E, 7742176.54S, elevation 722 m), located in the municipal district of Pains, Minas Gerais, Brazil. The caves have permanent groundwater and only Brega cave receives also water from rain floods.

The Brega Cave had predominantly vadose genesis, presenting a horizontal development of approximately 760 meters and two opposite entrances. Santuário Cave had also predominantly vadose genesis and presents a horizontal development of approximately 700 meters and two entrances. At the end of the main conduits of the two caves are small perennial water holes feed by upwelling groundwater.

According to the classification of Köppen (Menegasse et al. 2002), the climate of the external area is the Cwa type: mild climate with hot, humid summer and dry winter. The average annual temperature is 20.7°C, July being the coldest month, with an average temperature of 16.3°C, and January the hottest month, with an average of 23.3°C. The local annual average precipitation is 1344 mm. The Brega and Santuário caves belong to the hydrographic sub-basin known as *ribeirão dos Patos* (530 km<sup>2</sup>), favored by the direct infiltration in the limestone fissures and the karstic absorption features such as dolines and sinkholes.

The local tropical climate is responsible for the seasonal variation of the hydric dynamics of the *ribeirão dos Patos*, significantly altering the surrounding landscapes. The wet season lasts from October to March, which according to Menegasse et al. (2002) corresponds to 81% of the annual precipitation. The great hydric volume of the *Patos* stream makes the increase of the water level present inside the caves possible, leaving a large part of their floors flooded.

### Litter colonization and breakdown rates

Rates of litter breakdown in lentic environments were measured using bags containing 50 intact plant leaf disks (area = 63.6 mm<sup>2</sup>/each disk,  $\pm$  2 grams). The bags with 0.04 mm<sup>2</sup> and 9 mm<sup>2</sup> meshes, measuring 10  $\times$  10 cm<sup>2</sup> on the sides, enable the microinvertebrates colonization (Bärlocher 2005; Venarsky et al. 2012). 42 bags were used in each site, 21 with the smaller mesh size and 21 with the larger mesh size, totaling 84 litter bags in the two caves.

The leaves were collected from trees (Moraceae: *Ficus* sp.) located in the surrounding area before the fall of leaves, thereby reducing the possibility that the experimental material had been previously exposed to decomposition after falling on the ground.

### Sample processing

Triplicates of each mesh size were removed (after intervals of 14, 35, 50, 64, 78, 85 and 99 days) to quantify the decomposition through the weight loss and to determine the composition of the associated invertebrate fauna. Before the incubation of the litter bags in the cave water, the initial weight and the initial nitrate and phosphate levels present in the foliar portions were measured. After each collection, the material was



washed with distilled water (Bärlocher 2005), the wash water and the removed disks were conditioned separately in Petri dishes and observed with the aid of a magnifying glass, to verify the presence of associated invertebrates.

The remaining plant material and the wash water were oven-dried at 100° C for 48 hours and later weighed. After the material was dried and weighed, it was sent to the Plant Nutrition Laboratory of the Federal University of Paraná, Brazil, where the analysis of remaining nitrogen and phosphorous in the fractions under decomposition was conducted. Total leaf nitrogen and phosphorus were also measured, using the microKjeldahl method on another portion of the leaf (Varley 1966).

To evaluate the influence of the moisture of the green leaves on the weight loss measurements, 50 green leaves were weighed (2.03 gr), oven-dried and reweighed (0.48 gr). Thus, the 76.3% plant disk weight loss is due to the moisture loss and not the mass loss. Water samples were collected from each cave on the sampling start date and analyzed for turbidity, dissolved oxygen, phosphorus and nitrogen.

## Data analyses

The amount of organic matter remaining after exposure was expressed in percentage (%) of remaining dry weight  $[(FW \times 100)/IW]$ . Where IW is the initial weight of the sample at time zero, FW is the final weight of the sample at time  $t+1$ . The decay processing rate ( $k^{-day}$ ) in each cave was described by the model  $M_t = M_0 e^{-Kt}$  (Osion 1963; Bärlocher 2005), Where  $M_t$  is the weight at time  $t$ ,  $M_0$  is the initial weight;  $t$  is the exposure time of the sample.

To determine the processing speed ( $k/day$ ) of plant debris we compared our results to data from the literature for aquatic environments and defined slow ( $\leq 0.005$ ), moderate (0.006–0.10) and fast (0.10–0.15) processing (Table 1). To evaluate differences in the average final weight values among sites and among the dates of collection and in the same different mesh types, the  $t$  Test was used (Zar 1984).

## Results

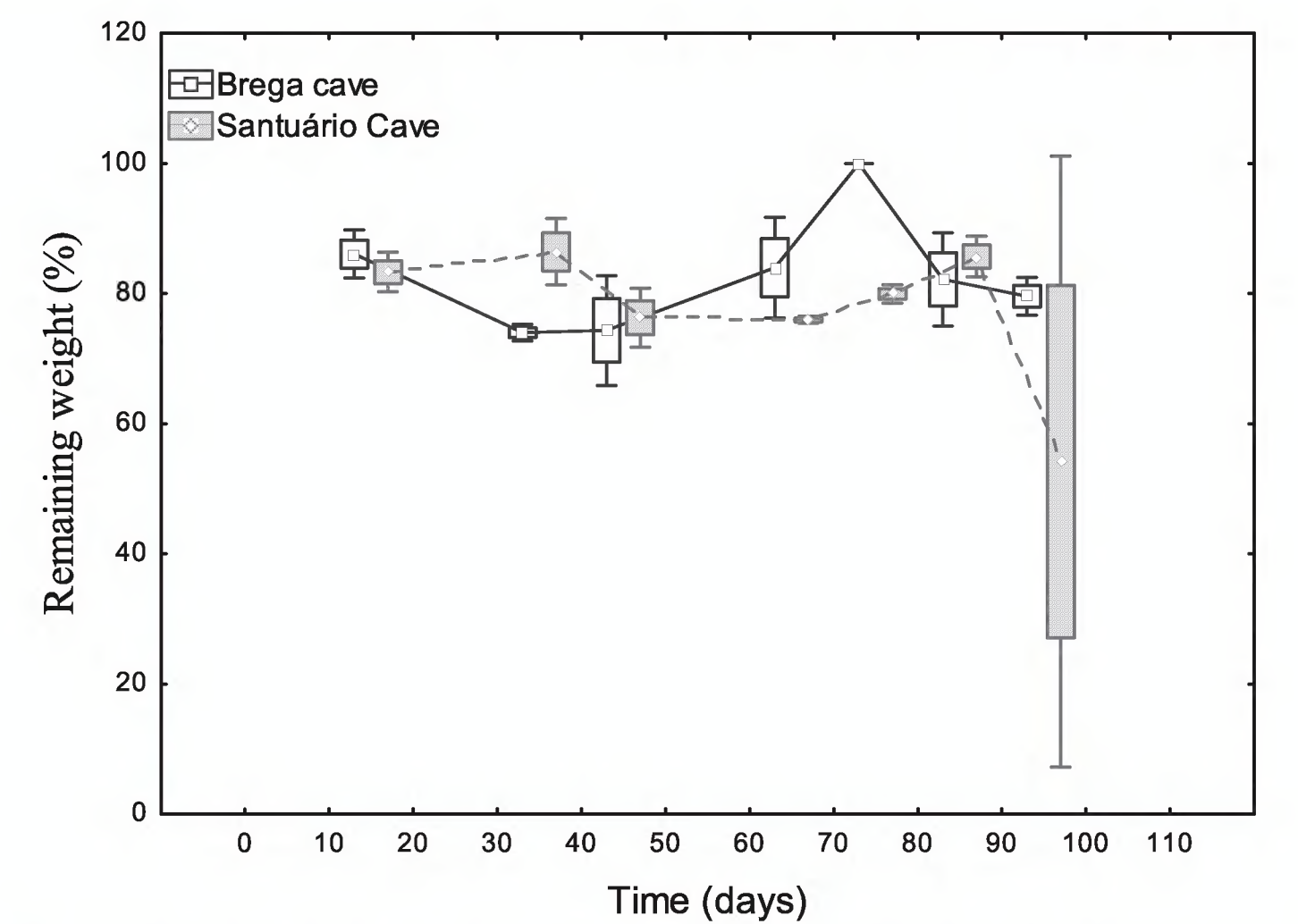
### Litter colonization and breakdown rates

The plant disks exposed to decomposition presented slow processing speed, but varied with time and between the two exposure caves (Table 1, Figs 1 and 2). However, significant differences were observed in the average final weight between both the two caves and the two mesh sizes (Table 2).

In Brega Cave, during the first 14 days of exposure of the 0.04 mm<sup>2</sup> mesh an average remaining weight of 86% was observed. After 85 days of exposure the plant debris had an average remaining weight of 82% in relation to the initial mass (Fig. 1). In the

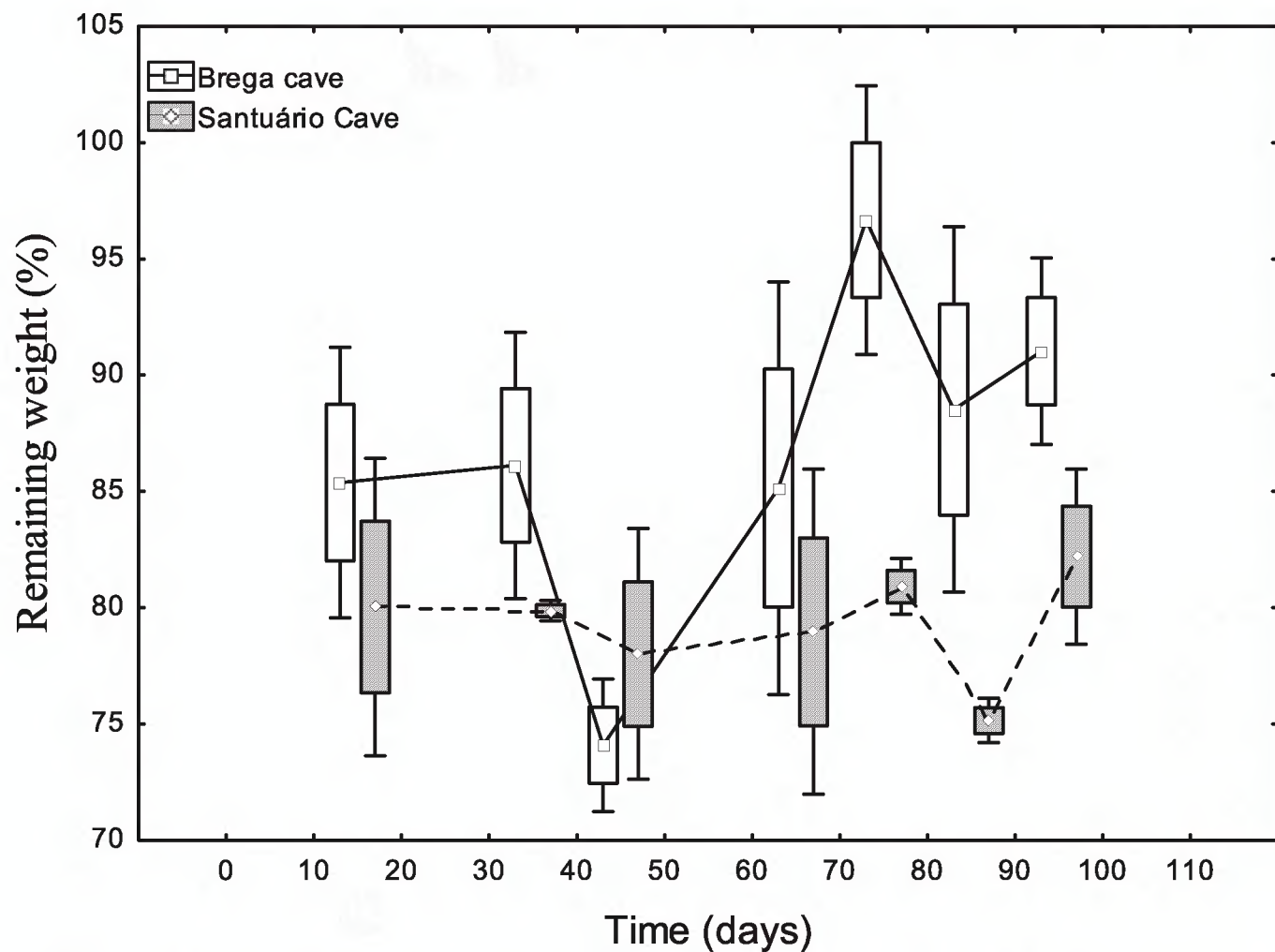
**Table 1.** Decay process ( $k^{-day}$ ) in this study compared to other published work from aquatic environments: Standard error (se).

Habitat type	$k^{-day}$	se	References
Brega cave water (mesh size 0.04 mm <sup>2</sup> )	0.0025	0.0005	This study
Brega cave water (mesh size 9 mm <sup>2</sup> )	0.002	0.004	This study
Santuário cave water (mesh size 0.04 mm <sup>2</sup> )	0.003	0.000	This study
Santuário cave water (mesh size 9 mm <sup>2</sup> )	0.0032	0.000	This study
Cave pool	0.003–0.001	-	Galas et al. 1996
Ephemeral river pool	0.009	-	Maamri et al. 1997
Littoral zone, Lake	0.0058–0.0039	-	Pope et al. 1999
Chaney Lake	0.0025	-	Kelley and Jack, 2002
Cave stream	0.0158	-	Simon and Benfield 2001
Cave stream	0.003	-	Simon and Benfield 2001
Cave stream	0.043–0.598	-	Souza-Silva et al. 2011
Cave stream	0,03-0.05	-	Souza-Silva et al. 2007
Cave stream	0.0173	-	Souza-Silva et al. 2012
Cave stream	0.001–0.012		Venarsky et al. 2012
Cave stream (mesh size 10×8 mm)	0.004–0.012		Venarsky et al. 2012



**Figure 1.** Remaining weight of plant disks exposed to processing in the 0.04 mm<sup>2</sup> litterbag mesh size in the lentic habitats of Brega and Santuário caves. Mean, Box: Mean±SE, Whisker: Mean±SD





**Figure 2.** Remaining weight of plant disks exposed to processing in the 9 mm<sup>2</sup> litterbag mesh size in the lentic habitats of Brega and Santuário caves. Mean, Box: Mean±SE, Whisker: Mean±SD.

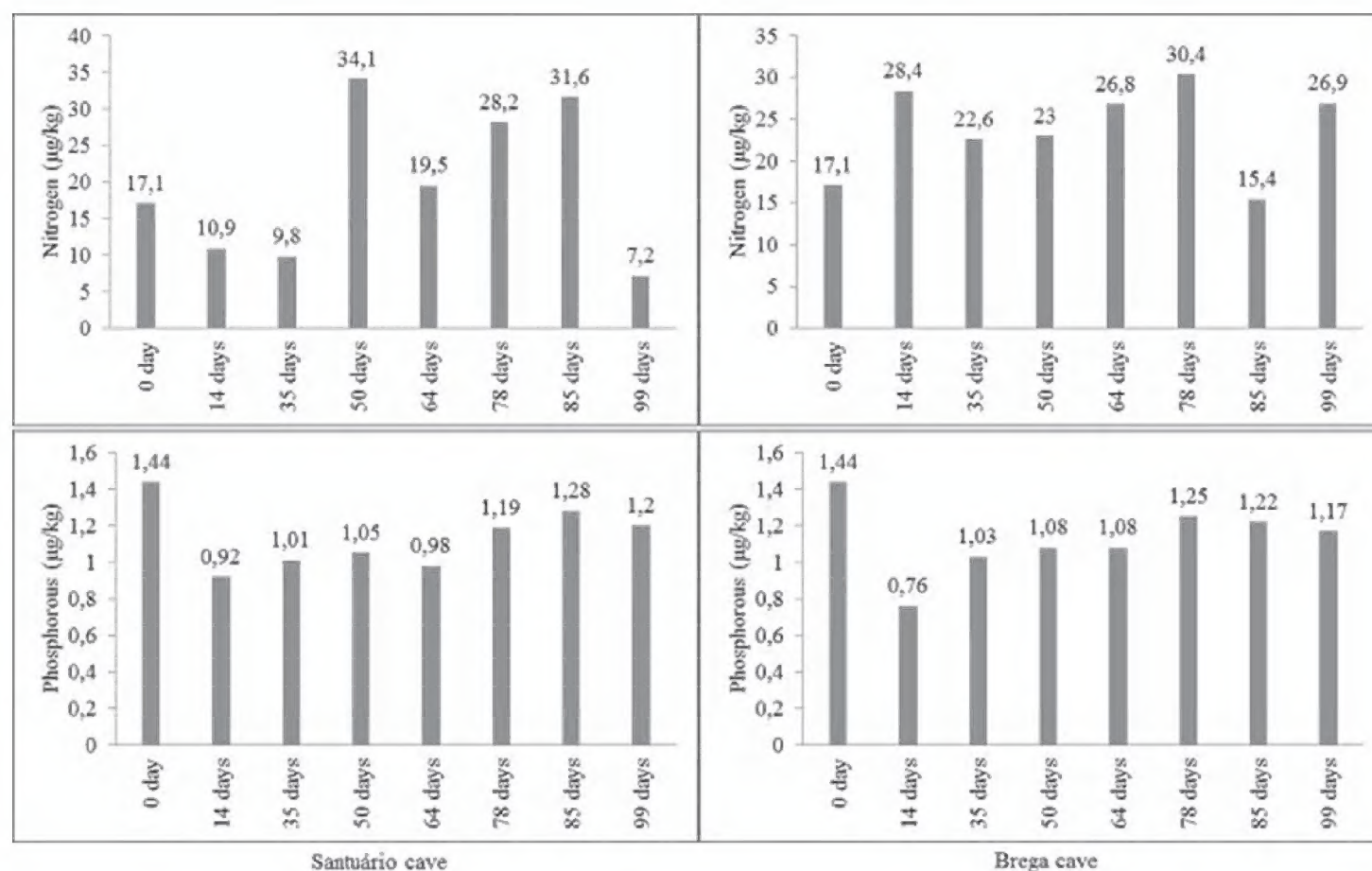
**Table 2.** Significant\* ( $p \leq 0.05$ ) and non-significant differences in the final plant matter weight processing between caves and litterbag mesh sizes in lentic environments: Brega Cave (B), Santuário Cave (S), 0.04 mm<sup>2</sup> mesh (1), 9 mm<sup>2</sup> (2).

Cave	Initial	Mean	Mean	t-value	df	p	Std.Dev.	Std.Dev.
mesh	weight	1	2				Group 1	Group 2
B2 x. S2*	0.5	0.461	0.417	4.313	40	0.000	0.042	0.021
B1 x. B2*	0.5	0.430	0.461	-2.171	40	0.036	0.051	0.042
B1 x. S1	0.5	0.430	0.419	0.855	39	0.398	0.051	0.027
B1 x. S2	0.5	0.430	0.417	1.060	40	0.296	0.051	0.021
S1 x. B2*	0.5	0.419	0.461	-3.845	39	0.000	0.027	0.042
S1 x. S2	0.5	0.419	0.417	0.248	39	0.805	0.027	0.021

same cave, during the first 14 days of the 9 mm<sup>2</sup> mesh plant disk exposures, an average remaining weight of 88% was observed. After 85 days of exposure of the plant disks, the average remaining weight corresponded to 88% (Fig. 2).

In Santuário Cave, during the 14 days of exposure in the 0.04 mm<sup>2</sup> mesh, an average remaining weight of 83% was observed. After 85 days of exposure of the plant disks, the average remaining weight was 84% (Fig. 1).

In Santuário Cave from the 14<sup>th</sup> to the 35<sup>th</sup> days of exposure of the 9 mm<sup>2</sup> mesh plant disks, an average remaining weight of 80% was observed. From the 78<sup>th</sup> to 85<sup>th</sup>



**Figure 3.** Temporal values of nitrogen and phosphorous ( $\mu\text{g/kg}$ ) measured in plant discs exposed to decomposition in lentic habitats of the Santuário and Brega caves.

days of exposure the average remaining weight was 78%. At the end of the 99 days of exposure, the average remaining weight corresponded to 82% (Fig. 2).

We can assume that for all of the mesh sizes a slow weight loss rate was registered. As such, we can also consider the low decay ( $\text{K/day}$ ) values (average  $0.003 \text{ K}^{-\text{day}}$ ) for the water of these two caves (Table 1).

There were no macroinvertebrates associated with the debris.

### Dynamics of nitrogen and phosphorus in the plant disks

The water quality parameters evaluated for the two caves showed quite different values. In Brega Cave the values were as follows: turbidity (6.97 unt), dissolved oxygen (5 mg/l), phosphorus (1.2 mg/l) and nitrogen (0.8 mg/l). In Santuário Cave the values were as follows: turbidity (39.5 unt), dissolved oxygen (4.5 mg/l), phosphorous (1.8 mg/l) and nitrogen (1.4 mg/l).

The values for nitrogen and phosphorous in the plant disks in the two caves showed different values during the experiment. However, significant differences were not found in the average amount of nitrogen and phosphorous present in the debris of the litterbags of the two caves.

In Santuário Cave, the initial nitrogen value was  $17.1 \mu\text{g/kg}$ . After 14 days of plant disk exposure (April 18) this value reduced to  $10.9 \mu\text{g/kg}$ . After 99 days of exposure (July 12) the lowest value ( $7.2 \mu\text{g/kg}$ ) of the remaining nitrogen was registered (Fig. 3). In Bre-



ga Cave, the initial nitrogen value was also of 17.1  $\mu\text{g/kg}$ . With 14 days of exposure (April 18), the amount of nitrogen in the plant disks increased (28.4  $\mu\text{g/kg}$ ). On the 99<sup>th</sup> day of exposure of the plant debris (July 12), the nitrogen value was of 26.9  $\mu\text{g/kg}$  (Fig. 3).

In Santuário Cave, the initial value of phosphorous was 1.44  $\mu\text{g/kg}$ . After 14 days of exposure of the plant debris (April 18) the lowest amount of this nutrient was registered, corresponding to 0.76  $\mu\text{g/kg}$ . After 85 days (June 28) there was a small reduction in the amount of phosphorous in the plant debris (Fig. 3). In Brega Cave, the initial phosphorous value was also of 1.44  $\mu\text{g/kg}$ . After 14 days of exposure (April 18) the lowest amount of this nutrient was registered, 0.92  $\mu\text{g/kg}$ . On the 99<sup>th</sup> of exposure (July 12), the amount of the remaining phosphorous in the plant debris showed a reduction to 1.20  $\mu\text{g/kg}$  (Fig. 3).

## Discussion

Works related to processing of plant debris have not been undertaken in lentic cave habitats until present. Therefore, the data of this study are compared with organic plant matter processing studies conducted in underground and surface streams, ephemeral karst lakes and oligotrophic lakes (Table 1). Some studies revealed that decomposition had different speeds, from slow to fast, in subterranean streams (Table 1).

Organic matter decomposition depends on the action of organisms, physical-chemistry features and abrasive force of the water, all playing an important role in the organic plant matter degradation (Allan and Castillo 2007). Usually in streams, the processing is faster if there is a combination of abrasive physical, chemical and biological factors such as pH, currents and shredders that accelerate the decomposition (Suberkropp et al. 1976; Allan and Castillo 2007).

The microorganisms and aquatic shredder invertebrates activity significantly interferes in the organic matter decomposition coefficient, constituting an indirect measure of the use potential of the debris as a food resource (Dobson et al. 2003). Shredders invertebrates, such as some amphipods, plecopterans, trichopterans, and dipterans, are important for leaf breakdown in European and North American streams (Graça 2001; Wantzen and Wagner 2006). However, in the tropical areas the shredder action can be slower when compared to the temperate areas (Irons et al. 1994; Moretti 2005, Wantzen and Wagner 2006). This difference can be due to highest shredder abundance and low temperatures in the temperate streams and the high palatability of the organic plant debris (thin cuticle and few toxic compounds) that favor the shredder activity over that of the microorganisms (Wantzen and Wagner 2006). During the decomposition processes, bacteria, fungi and Protozoa colonize and proliferate on the plant debris substrate. They play an important role in altering the palatability of leaves for scavengers and in fragmentation of debris (Arsuffi and Suberkropp 1984).

The slow decomposition rates of the organic plant matter in the lentic environments of Brega and Santuário caves due mainly to the fact that these environments are spatially and trophically restrictive to colonization by shredder invertebrates. Furthermore, the abrasive force of the water, which plays an important role in debris process-



ing and availability, is absent. Thus, lentic caves habitats can be vulnerable to organic matter enrichment when compared to surface aquatic environments or underground streams (Sket, 1999; Moretti 2005; Hahn 2006; Wantzen and Wagner 2006; Souza-Silva et al. 2011; Venarsky et al. 2012).

Caves and their microhabitats are often contaminated by nutrients resulting from human activities that are carried by water to the subterranean environment (Bowles and Arsuffi 1993; Simon and Buikema 1997; Gunn et al. 2000; Pronk et al. 2009; Souza-Silva et al. 2012). Many natural factors can also contribute to high input of organic matter in caves, such as landscape topology, surrounds plant community structure, cave depth, entrance size, fractures in the surrounding bedrock and climate (e.g. precipitation and hydrology) (Souza-Silva et al. 2012; Venarsky et al. 2012).

In cave aquatic habitats the quantity of organic matter is known as one factor that influences microorganisms and invertebrate fauna structure and physical-chemistry conditions (Simon and Buikema 1997; Wood et al. 2002; Graening and Brown 2003; Wood 2008).

The delay in the reduction of the amount of the nitrogen and phosphorous in the plant disks can be due to their low solubility and delay in colonization by microorganisms (Gessner 1991). On the other hand, the remaining nitrogen and phosphorous amount increase is probably due to subsequent colonization by microorganisms (Suberkropp and Klug 1976; Rogers and Debruyne 1998; Kelley and Jack 2002; Allan and Castillo 2007), knowing that water sources in caves provide a more suitable habitat for bacteria, fungi and protozoa communities (Mulec 2008; Sigalada -Regalado et al. 2011; Souza-Silva et al. 2012).

More researches are needed to understand how litter decomposition can be affected both by hydrology and biological communities. The understanding of the debris processing dynamics in the ecosystems and their influence on community structuring is fundamental for the biodiversity conservation in as much these processes represent the energy base of the systems.

## Acknowledgements

The authors are grateful to John Holsinger for English corrections of the manuscript and two anonymous reviewers for their useful suggestions. We express our thanks to the Coordenadoria de Pesquisa do Centro Universitário de Lavras (UNILAVRAS). Leopoldo Ferreira de Oliveira Bernardi, Mr. Brega (proprietário da fazenda), Rosinei (Coelho), Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo a pesquisa do Estado de Minas Gerais (Fapemig), Centro Nacional de pesquisa e conservação de cavernas (CECAV).

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